




## Article

# Mineral and Citrate Concentrations in Milk Are Affected by Seasons, Stage of Lactation and Management Practices

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Received: 27 December 2018; Accepted: 21 January 2019; Published: 29 January 2019



**Abstract:** This study was conducted to examine associations between nutrition, time of year and season of calving on milk mineral concentrations in 24 pasture-based dairy farms. There was substantial variation in the concentrations (mean with range in parentheses) of Ca 1072 (864–1310) mg/kg; citrate 1579 (880–2080) mg/kg; P 885 (640–1040) mg/kg; Mg 98 (73–122) mg/kg; Na 347 (248–554) mg/kg; K 1534 (1250–2010) mg/kg; and S 295 (155–372) mg/kg with most of the variation associated with stage of lactation, although the influence of days in milk was different for different minerals. Feeding practices were also important in determining the concentrations of some components. Milk Ca, citrate, P, and K concentrations were greater ( $p < 0.05$ ) in cows receiving mineral supplements, while Ca, P and Mg were positively correlated ( $p < 0.05$ ) with the amount of concentrates fed. Milk citrate and K concentrations were positively correlated ( $p < 0.05$ ) with herbage allowance. Milk Se (15 (<3–37)  $\mu\text{g/kg}$ ) and Zn (3.4 (2.2–4.9) mg/kg) also varied with time of year, with Se concentrations also greater (13.7 vs 11.6  $\mu\text{g/kg}$ ) in spring compared with autumn calving herds and positively correlated ( $p < 0.05$ ) with the amount of concentrates fed and mineral supplementation. These data indicate that calving pattern and feeding practices could be used to reduce variation in the measured milk mineral concentrations.

**Keywords:** lactation; milk quality; calcium; selenium; minerals

## 1. Introduction

In pasture-based dairy productions systems, stage of lactation, nutrition, and management decisions are known to affect the production and composition of milk protein and fat [1–4]. While seasonal spring calving used to be the predominant practice in northern Victoria, industry statistics indicate that the peak to trough ratio in milk supply in Victoria is now less than 2:1 [5]. There has been a substantial shift to calving some cows in spring and others in autumn (termed split calving), which has been attributed to seasonal price incentives, the failure of cows to conceive, improved knowledge of feeding, and use of supplements, as well as a sequence of dry winters [6].

Large fluctuations in the composition of milk supplied to dairy factories can increase processing costs [6–8] through impacts on the efficiency of processing into dairy products and the suitability of the milk for use as ingredients by the food industry (see [6,9–11]). Variations in milk mineral concentrations, like variation in milk protein and fat composition, affect suitability for processing.

For example, physical functional properties for manufacturing cheese and yoghurt are affected by the relative concentrations of Ca, P, Mg, and citrate [12–15], while physiological functional properties for human health can be influenced by concentrations of Zn and Se-rich proteins [16–19]. Dairy companies require sufficient knowledge of changes in the composition of milk over time to optimize processing efficiency and product mix and quality.

There is little information from any of the Australian dairy regions of Victoria on variations in the concentrations of minerals and citrate in milk throughout the year. This study was conducted to examine changes in the mineral concentrations in milk from farms in northern Victoria as affected by time of year (seasonal variation), season of calving (SOC), stage of lactation (SOL) and management practices.

## 2. Materials and Methods

### 2.1. Experimental Design

Twenty-four farms that calved in both spring and autumn as well as being representative of dairy farms in northern Victoria were selected for the study. These farms were also selected because they covered the range in amounts of grain fed from less than 0.5 to about 2.5 t grain/cow.lactation [3].

The experimental design allowed the assessment of associations between month, SOC, nutrition and grazing management on milk yield, and composition under commercial conditions. As it was logistically possible to only visit and sample 3 farms within any week, the 24 farms were sampled as 2 groups of 12. The farms in the first group were sampled in blocks of 4 weeks during April, July and October 2001 and January 2002, while those in the second group were sampled in blocks of 4 weeks from mid-May to mid-June, mid-August to mid-September and mid-November to mid-December in 2001 and mid-February to mid-March in 2002. The full experimental design is described in Reference [3].

For each farm, 16 Holstein Friesian cows from those that calved from late February to late April 2001 (Autumn calving) and 16 Holstein Friesian cows that calved from mid-August to late September 2000 (Spring calving) to values for the corresponding herd based on the most recently available herd records. As the sampling period straddled 2 lactations for the herds that calved in spring, an additional group of 16 cows that calved from mid-August to late September 2001 were selected so as to be representative of the cows calving in Spring on each farm. Cows were not selected if they had high somatic cell counts. Using these selection criteria, the association between time of year and milk mineral concentration could be described at 8 points ~ 6–7 weeks apart over a 12-month collection period, and the association between SOC and SOL on milk composition could be described at 7 points ~ 6–7 weeks apart, across a lactation of ~42 weeks. Milk samples were not taken from the spring-calving herds in the July sampling period and the autumn-calving herds in the February–March sampling period when cows were dry. Associations between nutrition or grazing management and milk composition were determined after first accounting for the effects of SOC and SOL.

### 2.2. Nutrition and Grazing Management

The area of pasture available to the combined herd within an irrigation bay (small paddock on irrigated dairy farms that is flood irrigated) for strip grazing prior to and after the evening milking was measured at each farm visit. This was assumed to be representative of the herbage available after both the evening and morning milking, and no measurements were made on pasture offered after the morning milking. A rising plate meter [20] was used to measure the height of pasture (the average of 100 plate meter readings) that was offered to cows after the evening milking. The average heights of perennial pastures were converted to pre-grazing pasture mass using the Dietcheck program [21]. Average plate meter heights of annual pastures (subterranean or Persian clovers) were converted to pre-grazing pasture mass using relationships reported for studies conducted in northern Victoria for subterranean clover [22] or Persian clover [23] pastures. Unless visual assessment suggested otherwise, it was assumed that the remaining pasture within a bay (that was not available to the herd) was

representative of the pre-grazing pasture mass within that bay. Average daily herbage allowance per cow was calculated from the estimates of pasture mass, the area available for grazing, and the number of cows in the milking herd (autumn + spring-calved cows). Pasture mass was only estimated when the pasture was not wet from rain and herbage allowance was estimated only when strip grazing was being used. Strip grazing was not being used on 6 out of the 24 farm visits made in May–June and July (late autumn and winter) and so herbage allowance could not be calculated. However, pasture mass data collected on these visits was included in the statistical analysis.

The botanical composition of herbage available was estimated by visually determining the proportions of paspalum, ryegrass, clover, weeds, and dead material within 6 quadrats (0.245 m<sup>2</sup>) within each grazing area and an average obtained. The ME content of herbage consumed from perennial pastures was estimated using the Dietcheck program [21]. The ME content of herbage consumed from annual pastures during their growing season was assumed to be the maximum value recorded for perennial pastures at the same time of year within the Dietcheck program. Concentrate supplements were generally offered in the bail during the morning and evening milkings except for 6 out of the 96 farm visits, where concentrates were fed in both the bail during milking and as a component of a mixed ration after milking. Where concentrates were fed as part of a mixed ration, the farmer specified the composition of the mixed ration. Since concentrates and mixed rations were measured by volume, known volumes of concentrate and mixed rations were weighed on each farm visit to determine amount fed. Information was also collected on the composition of concentrate supplements, the form in which they were fed, and whether they contained mineral supplements (Table 1). The macro-mineral supplements and complete mineral mixes were all high in Ca. Farmers provided estimates of the amounts of conserved forages fed, but no measurements were made of the weights of forage supplements offered or of wastage of those supplements so that statistical analyses were for amounts of forages offered rather than amounts consumed.

**Table 1.** Numbers of farms in four categories for amounts of concentrates fed and within those categories for the type of concentrate and whether mineral supplements were used.

Parameter	Concentrates Fed (kg DM/cow.day)			
	0–3	>3–6	>6–9	>9
Number of farms	7	7	8	2
Herd size (average for category) <sup>1</sup>	100–280 (200)	130–280 (207)	100–1150 (463)	210–210 (210)
Concentrate Type <sup>2</sup>	2 grain/2 mix	2 grain/5 mix	2 grain/6 mix	0 grain/2 mix
Macro-mineral supplement fed	4 no/3 yes	0 no/7 yes	0 no/8 yes	0 no/2 yes
Trace element supplement fed	5 no/2 yes	2 no/5 yes	2 no/6 yes	0 no/2 yes

<sup>1</sup> Herd size refers to the maximum number of cows milked daily over the 12 months of sample collection. <sup>2</sup> Grain refers to cracked or hammer-milled cereal grains (usually barley, wheat, and/or triticale). Mix refers to a proprietary feed mix supplied by a commercial feed company based on cereal grains, protein meals, vegetable oils, and/or oil seeds.

### 2.3. Milk Production and Composition

A representative sample of milk (22 g sample/kg milk) was obtained at the afternoon and subsequent morning milking at each farm visit from the 16 focus cows for each calving group before pooling to obtain a composite sample of milk for analysis for each seasonal calving groups. The pooled samples were preserved with preservative (ca. 0.045% W/W Bronopol; CAS number: 52-51-7) and placed in a refrigerator (3–4 °C) before analysis within 7 days.

The concentration of crude protein was determined using a CombiFoss 5000 (i.e., linked Milkoscan + Fossmatic; Foss Electric, Denmark). Triplicate samples were analysed using a Leco FP-2000 combustion nitrogen analyzer (LECO Australia Pty Ltd, Castle Hill, Australia) for total nitrogen, true protein nitrogen following isolation of the protein by precipitation with 12% trichloroacetic acid [24], and casein nitrogen following isolation of the protein by precipitation at pH 4.6 according to the method of Reference [25] with modifications as described by Reference [3].

Milk samples for determination of concentrations of Ca, Mg, K, Na, P, S, and Zn were homogenized by vigorous shaking. Three grams of milk were weighed into a digestion vessel, sealed, and digested in a mixture of nitric acid and hydrogen peroxide using microwave acid digestion to 180 °C for 15 min. The digestate was diluted to 40 mL prior to analysis using Inductively Coupled Plasma Emission Spectroscopy (ICP-ES, Spectro-Flame-EOP, Spectro Analytical Instruments GmbH, Kleve, Germany) for the determination of Ca, Mg, K, Na, P, and S or Atomic Absorption Spectrophotometry (Varian SpectrAA-300/400, Varian, Mulgrave, Australia) for the determination of Zn. The limits of detection and resolution were 1 and 3 mg/kg, respectively for Ca, K, and Na; 0.7 and 3 mg/kg for Mg, 4 and 9 mg/kg for P, 7 and 21 mg/kg for S, and 0.5 and 2 mg/kg for Zn.

Total Se in milk samples was determined after digesting subsamples in a mixture of nitric and perchloric acids by using test tubes fitted with reflux funnels in an electrically heated digestion block. In the end stage of the digestion process, hydrochloric acid was added to the tubes to reduce Se (V) to Se (III), with the final matrix being 30% hydrochloric acid. The digestate was diluted (to 20 mL final volume) and analysed by Inductively Coupled Plasma Mass Spectrometry/Vapour Generation (ICP-MS, Ultramass ICP-MS, Varian, Mulgrave, Australia). Duplicate samples were included at 3–4 per 100 samples, and reproducibility was at least 90%. Standard reference material (Institute of Standards and Technology standard 1549—non-fat milk powder) and laboratory control standards were carried through all analyses. In each run, two randomly selected samples were spiked, with acceptable recoveries being between 80 and 120%. The limits of detection and resolution were 1 and 3 µg/kg.

Citrate/citric acid was determined using a commercial assay kit (Boehringer Mannheim Citrate/citric acid kit Cat 139076) following deproteinization of the sample with Perchloric acid (HClO<sub>4</sub>): A test portion (~0.75 mL) was weighed (to 0.0001 g) into a standard mini-centrifuge tube and ice cold HClO<sub>4</sub> solution (1 M 0.75 mL) added. The weight of the total mixture was recorded. The precipitated protein was pelleted by centrifugation (14,000× g/10 min) and an aliquot of the supernatant (approximately 0.75 mL weighed accurately), removed and mixed with a solution of K<sub>2</sub>CO<sub>3</sub> (0.3 M, 0.75 mL), and incubated at 4 °C for 10 min. The weight of the total mixture was recorded. The samples were clarified by centrifugation, and duplicate aliquots (0.2 mL each) were removed for assay according to the manufacturer's instructions.

#### 2.4. Statistical Analysis

Statistical analysis for the effects of nutrition and management on milk fat components was by analysis of variance using Residual Maximum Likelihood (Genstat Version 11, release 11.1.0.1575, VSN International, Hemel Hemstead, United Kingdom). Definitions of the terms used are provided in Table 2. All models contained a random effects term to account for the effect of a particular farm (Farm: 1 to 24) and a visit at a particular time to that farm (Visit: 1 to 4). The effects of SOC at a particular period of sampling (fixed effect) were tested for main and interactive effects. The effects of time of calving at a particular SOL (fixed effect) were tested using a model that included the main and interactive effects of SOC and SOL. The effects of variation in nutritional regimen or grazing management were tested using a model that included the interactive effects between SOC and SOL and nutritional regimen or grazing management [3]. The effects of the estimated ME content of herbage consumed, amount of conserved forage offered, and herbage allowance were tested for each analyte using simple linear regression, as these factors were seen to co-vary with both time of year and SOL. Summary statistics to describe the between- farm variation in milk components for both the autumn and spring calving groups were calculated using the average value for all samples from each calving group within a farm (Table 3).

**Table 2.** Descriptors used to define the management structure of dairy farms in the study <sup>1</sup>.

Descriptors	Factor/Variate	Definition
Structural Components		
Farm	Factor	Farm identifier (1 to 24).
Calving group	Factor	Calving group: 1 = autumn, 2 = spring.
DIM <sup>2</sup>	Variate	Average days in milk as a discrete variable calculated for each calving group within a farm at a particular visit.
Period	Factor	Period (4 weeks duration) in which samples were collected (1 = Apr, 2 = May/Jun, 3 = Jul, 4 = Aug/Sep, 5 = Oct, 6 = Nov/Dec, 7 = Jan, 8 = Feb/Mar).
Stage of lactation	Factor	Stage of lactation as a category calculated as the average days <i>post-partum</i> for each herd on all farms visited during a sampling period (1 = 24, 2 = 60, 3 = 113, 4 = 145, 5 = 199, 6 = 231 and 7 = 288 days <i>post-partum</i> , respectively).
Visit	Factor	Visit to a particular farm (1 to 4).
Inputs		
Calcium supplement	Factor	Was a calcium supplement fed: yes = 1, no = 0
Concentrate	Variate	Amount of concentrate (grain or grain mix) fed (kg DM/cow·day)
Conserved forage	Variate	Amount of silage and/or hay fed (kg DM/cow·day)
Herbage allowance	Variate	Herbage allowance (kg DM/cow·day)
Herbage ME	Variate	Estimated metabolizable energy in herbage consumed (MJ ME/kg DM)
Size	Variate	Number of cows milked on the day of sampling
Trace supplement	Factor	Was a trace mineral supplement fed: yes = 1, no = 0

<sup>1</sup> Descriptors are divided into two groups, those aspects of production that are defined by the design of the study (structural) and those that are inputs to the farm production system (inputs). <sup>2</sup> DIM refers to days in milk.

**Table 3.** Statistics summarizing the effects of autumn or spring calving, when averaged across all farms, on the yield, concentrations of protein fractions, minerals and citrate in milk <sup>1</sup>.

Title	Autumn				Spring			
Component	mean	c.v.%	min.	max.	mean	c.v.%	min.	max.
Gross Composition								
Milk yield	24.5	15	15.9	29.9	23.6	21	17.7	34.8
Crude protein	33.1	3.3	26.7	37.4	34.1	3.5	30.2	41.3
True protein	30.6	4.6	24.8	36.2	31.5	3.9	27.2	37.7
Casein protein	25.0	4.8	20.2	28.9	25.8	4.0	22.3	31.1
Minerals and Citrate								
Ca	1070	8	891	1310	1080	8	864	1290
Mg	98	9	82	122	98	11	73	118
P	891	7	690	1020	879	9	640	1040
S	294	11	155	348	300	10	191	372
Se	11.6	45	3.0	30.3	13.7	51	1.7	37.1
Zn	3.45	16	2.15	4.91	3.48	15	2.52	4.73
Na	346	11	278	461	355	19	248	554
K	1540	9	1300	1870	1520	12	1250	2010
Citrate	1580	15	880	2040	1570	14	960	2080

<sup>1</sup> Milk yield has units of kg/cow.day, while milk protein concentrations have units of g/kg milk and milk mineral concentrations have units of mg/kg milk with the exception of Se where units are µg/kg milk.

Data from the present study and a number of published studies where both milk Ca and protein were reported were subjected to regression analyses to determine the relationship between the two milk constituents using the Generalized Linear Model Procedure in Genstat. Published studies were identified using PubMed and Web of Science databases using the key words calcium, protein, and

cow (or cattle). Only papers that reported milk protein and milk calcium content were included in the analyses, and there was no minimum number of observations required for inclusion. The initial model included breed, but when there were no effects of breed, the pooled response is presented.

### 3. Results

Summary statistics describing the concentrations of minerals and citrate in milk from herds that calved in autumn and spring are presented in Table 3. Relationships between milk production and gross composition and season, SOL or herd management have been reported by Reference [3]. However, the concentrations of crude protein and casein are reproduced in Table 3, as relationships between these fractions and concentrations of some minerals are examined below.

#### 3.1. Calcium and Magnesium

The average concentrations of Ca or Mg in milk did not differ significantly between SOC groups when averaged for SOL and the coefficients of variation, minimum, and maximum values for Ca or Mg in milk were similar for both SOC groups (Table 3). The concentration of Ca in milk varied little from 25 to 150 days postpartum, but increased ( $p < 0.05$ ) across the remainder of lactation with the highest concentration observed around 300 days postpartum (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and time of year, where herds that calved in spring produced milk with a higher concentration of Ca in May–June and a lower concentration in October and January (Table 4).

The concentration of Mg in milk increased at a similar rate from 25 to 300 days *post-partum* for both SOC groups, with the exception that herds that calved in spring produced milk with a higher ( $p < 0.05$ ) concentration of Mg around 150 days *post-partum* when compared with those that calved in autumn (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and time of year where herds that calved in spring produced milk with a higher concentration of Mg in April and May–June and a lower concentration in October, November–December, and January when compared with herds that calved in autumn (Table 4).

On average, the concentrations of Ca and Mg in milk increased ( $p < 0.05$ ) by  $7 \pm 2.5$  mg/kg and  $0.8 \pm 0.23$  mg/kg, respectively, for every extra kg DM of concentrates fed (Table 5) and that of Mg increased ( $p < 0.05$ ) by  $0.1 \pm 0.05$  mg/kg for every extra kg DM pasture offered. The concentration of Ca in milk did not vary with herbage allowance. The concentrations of Ca and Mg were higher ( $p < 0.05$ ;  $46 \pm 17.3$  and  $4 \pm 1.8$  mg/kg, respectively) in milk from herds receiving macro-mineral supplements (Table 6). The effect of feeding these minerals supplements on Ca concentration was apparent in the May–June and November–December sampling periods only (Figure 1a). Concentrations of Ca and Mg in milk did not vary significantly with estimated ME content of pasture or the amounts of conserved forages fed.

The ratio of Ca relative to casein varied by time of year for herds that were not fed high-Ca mineral supplements, with the ratio being lower ( $p < 0.05$ ) in April and November–December when compared with May–June, July, January and February–March. There was no significant variation in the ratio of Ca relative to casein by time of year for herds fed macro-mineral supplements. Herds fed macro-mineral supplements had higher Ca to casein than those which were not in April, October, and November–December (Figure 1b).

The ratio of Ca relative to citrate in milk varied by time of year for herds that were not fed high-Ca supplements, being higher in January when compared with all other sampling periods. There was no variation in the ratio of Ca relative to citrate by time of year for herds fed macro-mineral supplements and the supplemented herds produced milk with a lower ( $p < 0.05$ ) ratio of Ca to citrate in January (Figure 1c).



**Table 4.** The average concentrations of minerals and citrate in milk for herds that calved in autumn or spring by the time of year that samples were collected.

Time of Year									
Calving	April	May/Jun	July	Aug/Sep	Oct	Nov/Dec	Jan	Feb/Mar	s.e.d
Stage of lactation (days)									
Autumn	24	60	113	145	199	231	288	dry	-
Spring	231	288	dry	24	60	113	145	199	
Ca (mg/kg)									
Autumn	1086 <sup>def</sup>	1021 <sup>bcd</sup>	1044 <sup>cde</sup>	1010 <sup>bc</sup>	1218 <sup>h</sup>	971 <sup>ab</sup>	1145 <sup>fg</sup>	dry	35.3
Spring	1114 <sup>efg</sup>	1137 <sup>fg</sup>	dry	1057 <sup>cde</sup>	1158 <sup>gh</sup>	937 <sup>a</sup>	1101 <sup>efg</sup>	1057 <sup>cde</sup>	
Mg (mg/kg)									
Autumn	97 <sup>cde</sup>	91 <sup>abc</sup>	97 <sup>cde</sup>	91 <sup>abc</sup>	106 <sup>fgh</sup>	94 <sup>bcd</sup>	114 <sup>h</sup>	dry	3.8
Spring	107 <sup>gh</sup>	104 <sup>efg</sup>	dry	87 <sup>ab</sup>	98 <sup>cde</sup>	84 <sup>a</sup>	106 <sup>fgh</sup>	99 <sup>def</sup>	
P (mg/kg)									
Autumn	897 <sup>bcde</sup>	837 <sup>ab</sup>	919 <sup>def</sup>	889 <sup>bcd</sup>	976 <sup>f</sup>	818 <sup>a</sup>	906 <sup>cde</sup>	dry	29.7
Spring	838 <sup>ab</sup>	840 <sup>ab</sup>	dry	952 <sup>ef</sup>	975 <sup>f</sup>	804 <sup>a</sup>	894 <sup>bcde</sup>	851 <sup>abc</sup>	
S (mg/kg)									
Autumn	277 <sup>bc</sup>	246 <sup>a</sup>	292 <sup>bcd</sup>	296 <sup>cd</sup>	326 <sup>e</sup>	288 <sup>bcd</sup>	331 <sup>e</sup>	dry	12.2
Spring	315 <sup>de</sup>	313 <sup>de</sup>	dry	308 <sup>de</sup>	297 <sup>cd</sup>	267 <sup>ab</sup>	311 <sup>de</sup>	291 <sup>bcd</sup>	
Se (µg/kg)									
Autumn	14.5 <sup>bcd</sup>	13.2 <sup>abc</sup>	9.8 <sup>a</sup>	12.0 <sup>ab</sup>	9.9 <sup>a</sup>	9.8 <sup>a</sup>	12.2 <sup>ab</sup>	dry	2.21
Spring	16.9 <sup>cd</sup>	18.3 <sup>d</sup>	dry	14.0 <sup>abcd</sup>	10.1 <sup>ab</sup>	9.7 <sup>a</sup>	10.3 <sup>ab</sup>	16.9 <sup>cd</sup>	
Zn (mg/kg)									
Autumn	4.12 <sup>e</sup>	2.93 <sup>ab</sup>	2.73 <sup>a</sup>	3.64 <sup>cd</sup>	3.60 <sup>cd</sup>	3.70 <sup>cde</sup>	3.65 <sup>cd</sup>	dry	0.23
Spring	4.05 <sup>de</sup>	3.34 <sup>bc</sup>	dry	4.01 <sup>de</sup>	3.31 <sup>bc</sup>	3.46 <sup>c</sup>	3.33 <sup>bc</sup>	2.94 <sup>ab</sup>	
K (mg/kg)									
Autumn	1437 <sup>bc</sup>	1613 <sup>ef</sup>	1664 <sup>fg</sup>	1537 <sup>de</sup>	1612 <sup>ef</sup>	1503 <sup>cd</sup>	1425 <sup>abc</sup>	dry	49.0
Spring	1328 <sup>a</sup>	1373 <sup>ab</sup>	dry	1511 <sup>cd</sup>	1727 <sup>g</sup>	1566 <sup>de</sup>	1613 <sup>def</sup>	1557 <sup>de</sup>	
Na (mg/kg)									
Autumn	350 <sup>cd</sup>	334 <sup>bc</sup>	325 <sup>bc</sup>	326 <sup>bc</sup>	378 <sup>de</sup>	336 <sup>bc</sup>	379 <sup>de</sup>	dry	15.9
Spring	401 <sup>e</sup>	462 <sup>f</sup>	dry	317 <sup>b</sup>	328 <sup>bc</sup>	285 <sup>a</sup>	339 <sup>bc</sup>	353 <sup>cd</sup>	
Citrate (mg/kg)									
Autumn	1648 <sup>ab</sup>	1635 <sup>ab</sup>	1532 <sup>ab</sup>	1574 <sup>ab</sup>	1613 <sup>ab</sup>	1694 <sup>ab</sup>	1402 <sup>a</sup>	dry	121.7
Spring	1597 <sup>ab</sup>	1581 <sup>ab</sup>	dry	1516 <sup>ab</sup>	1555 <sup>ab</sup>	1661 <sup>ab</sup>	1420 <sup>a</sup>	1722 <sup>b</sup>	

Within mineral contents, means followed by different superscripts are significantly different at  $p = 0.05$ .

**Table 5.** Effect of dietary concentrate supplementation on milk mineral and citrate concentrations.

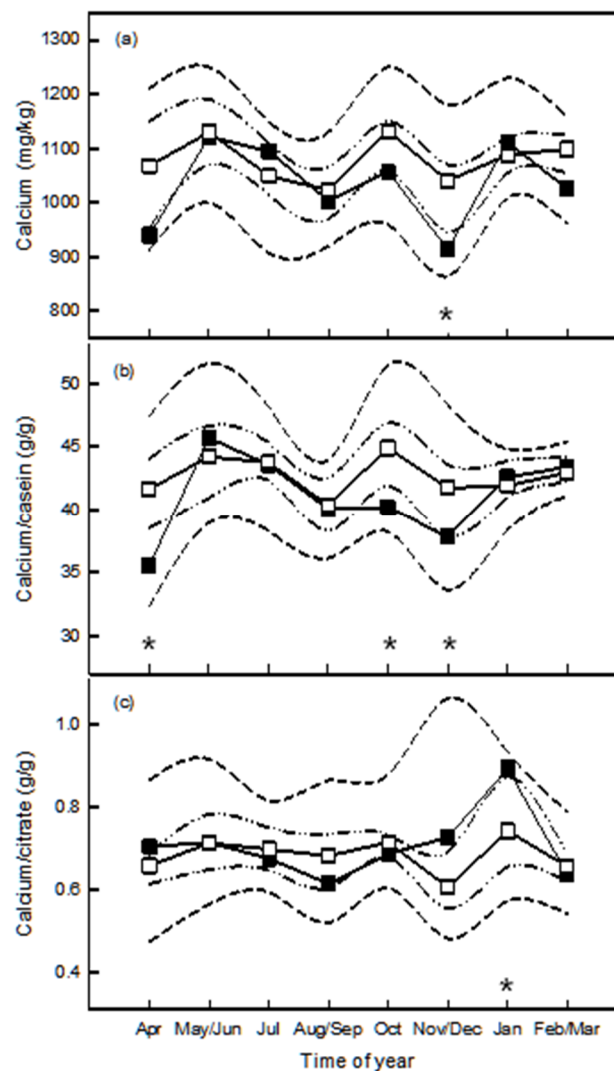
Mineral	Effect per kg Concentrates <sup>1</sup>	s.e.d.	p-Value
Ca (mg/kg)	7	2.5	<0.05
Mg (mg/kg)	0.8	0.23	<0.01
P (mg/kg)	7	2.0	<0.01
S (mg/kg)	-	-	NS
Se (µg/kg)	0.8	0.18	<0.01
Zn (mg/kg)	-	-	NS
K (mg/kg)	-	-	NS
Na (mg/kg)	-	-	NS
Citrate (mg/kg)	-	-	NS

<sup>1</sup> change in milk mineral or citrate concentrations per kg DM increase in reported concentrate supplementation.

**Table 6.** Effect of dietary mineral supplementation on milk mineral and citrate concentrations.

Mineral	Effect with Supplement <sup>1</sup>	s.e.d.	p-Value
Ca (mg/kg)	46	17.3	<0.05
Mg (mg/kg)	4	1.8	<0.05
P (mg/kg)	46	14.5	<0.05
S (mg/kg)	-	-	NS
Se (µg/kg)	7	1.3	<0.01
Zn (mg/kg)	-	-	NS
K (mg/kg)	-	-	NS
Na (mg/kg)	-	-	NS
Citrate (mg/kg)	114	53.8	<0.05

<sup>1</sup> change in milk mineral or citrate concentrations cows reported to receive vitamin/mineral supplementation.



**Figure 1.** The average concentration of (a) calcium in milk, (b) the ratio of calcium to casein and (c) ratio of calcium to citrate in milk from herds that either received (□) or did not receive (■) mineral supplements high in calcium by the time of year the sample was collected. An asterisk indicates a significant difference exists between herds receiving and not receiving a supplement of calcium at that time of year. Dashed lines indicate the minimum and maximum values observed at each period; dash-dot-dot lines indicate the lower and upper quartiles; all have been smoothed using a cubic spline function.



### 3.2. Citrate, Phosphorus and Sulphur

The average concentrations of citrate or P in milk did not differ between SOC groups when averaged for SOL and the coefficients of variation, minimum, and maximum values for citrate or P in milk were similar for both calving groups (Table 3). The concentration of S in milk was higher ( $p < 0.05$ ) for herds that calved in spring compared with herds that calved in autumn, although the difference was biologically small, while the coefficients of variation, minimum, and maximum values for S in milk were similar for both SOC groups.

For citrate, there was an interaction between SOC and SOL where the concentration was higher ( $p < 0.05$ ) in milk from herds that calved in spring around 100 and 300 days *post-partum*, and lower around 150 days *post-partum*, compared with herds that calved in autumn (Table 4). The concentration of citrate in milk did not vary much with time of year, averaged for SOC, except that milk citrate was lower in January than in November–December and February–April (Table 4).

For P, there was an interaction between SOL and SOC with the concentration declining in milk of herds that calved in spring across early- and mid-lactation and increasing across the same period for herds that calved in autumn (Table 4). The concentration of P in milk varied with time of year, being higher ( $p < 0.05$ ) for both calving groups in July, August–September and October compared with February–March and April (Table 4). There was an interaction between SOC group and time of year such that herds that calved in spring produced milk with a lower ( $p < 0.05$ ) concentration of P in April and higher ( $p < 0.05$ ) concentration of P in August–September, when compared with herds that calved in autumn.

The concentration of S in milk varied with SOL, declining from 25 to 50 days in milk and then increasing across the remainder of the lactation (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and SOL with herds that calved in spring producing milk with a higher concentration of S around 25 and 50 days *post-partum*. There was an interaction ( $p < 0.05$ ) between calving group and time of year, where herds that calved in spring produced milk with higher ( $p < 0.05$ ) concentrations of S in April and May/June when compared with herds that calved in autumn (Table 4).

The concentration of citrate was higher ( $p < 0.05$ ) in milk from herds receiving macro-mineral supplements ( $114 \pm 53.8$  mg/kg) (Table 6), but was not significantly affected by the estimated ME content of pasture or the amounts of concentrates fed. On average, the concentration of P in milk increased ( $p < 0.05$ ) by  $7 \pm 2.0$  mg/kg for every extra kg DM concentrates fed (Table 5), and was higher in milk from herds receiving macro-mineral supplements ( $46 \pm 14.5$ ;  $p < 0.05$ ) (Table 6). The concentration of S in milk increased ( $p < 0.05$ ) by  $9 \pm 3.2$  mg/kg for every extra MJ ME in pasture consumed, but was not affected by the amount of concentrates fed or mineral supplementation Tables 5 and 6). The concentrations of citrate, P or S in milk were not affected by herbage allowance or the amounts of conserved forages fed.

### 3.3. Selenium and Zinc

The concentration of Se in milk was higher ( $p < 0.05$ ) for herds that calved in spring compared with herds that calved in autumn, and the coefficient of variation was larger, minimum value smaller, and maximum value larger for Se in milk from spring calving compared with autumn calving herds (Table 3). There was an interaction ( $p < 0.05$ ) between calving group and SOL with herds that calved in spring, producing milk with a higher concentration of Se around 200, 225, and 300 days *post-partum* (Table 4). When averaged for the effect of SOC group, the concentration of Se in milk varied with time of year, being higher ( $p < 0.05$ ) in April and May–June compared with October and November/December. There was an interaction ( $p < 0.05$ ) between SOC group and time of year such that herds that calved in spring produced milk with higher ( $p < 0.05$ ) concentrations of Se in May–June when compared with herds that calved in autumn (Table 4).

The concentration of Zn in milk, averaged for SOL, did not differ significantly between calving groups and the coefficients of variation, minimum, and maximum values for Zn in milk were similar for both SOC groups (Table 3). There was an interaction ( $p < 0.05$ ) between SOC group and SOL

with herds that calved in spring, producing milk with a higher concentration of Zn around 100 days *post-partum* and a lower concentration around 150 days *post-partum* when compared to herds that calved in autumn (Table 4). When averaged for the effect of SOC group, the concentration of Zn in milk varied with time of year, being higher ( $p < 0.05$ ) in April and August–September when compared with July and February/March (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and time of year, so that herds that calved in spring produced milk with higher concentrations of Zn in May–June and August–September and lower concentrations in October, November–December, and January when compared with herds that calved in autumn.

On average, the concentration of Se in milk increased ( $p < 0.05$ ) by  $0.8 \pm 0.18$   $\mu\text{g}/\text{kg}$  for every extra kg DM concentrates fed (Table 5), and was higher ( $p < 0.05$ ) for herds fed macro-mineral ( $7 \pm 1.3$   $\mu\text{g}/\text{kg}$ ) (Table 6) and/or trace element ( $7 \pm 1.2$   $\mu\text{g}/\text{kg}$ ) supplements (data not shown). The concentration of Zn in milk did not vary significantly with these factors, and the concentrations of Se and Zn were not affected by herbage allowance, estimated ME content of herbage consumed, or the amounts of conserved forages fed.

### 3.4. Sodium and Potassium

The concentrations of Na or K in milk, averaged for SOL, did not differ significantly between SOC groups (Table 3). However, the coefficient of variation was larger, minimum value smaller, and maximum values larger for Na and K in milk for spring, compared with autumn calving herds (Table 3). The average concentration of Na in milk varied ( $p < 0.05$ ), with SOL tending to decline for both calving groups from 25 to around 100 days *postpartum* and then increase for the remainder of the lactation (Table 4). There was an interaction ( $p < 0.05$ ) between SOC and SOL, so that herds that calved in spring produced milk with a lower concentration of Na around 25 and 100 days *postpartum* and a higher concentration around 250 and 300 days *postpartum*. There was an interaction ( $p < 0.05$ ) between calving group and time of year, where herds that calved in spring produced milk with higher concentrations of Na in April and May/June and lower concentration in October, November–December and January (Table 4).

The average concentration of K in milk varied with SOL, increasing for both SOC groups from 25 to 50 days *post-partum* and then declining across the remainder of lactation (Table 4). There was an interaction ( $p < 0.05$ ) between SOC and SOL where herds that calved in spring produced milk with a higher concentration of K around 50 days *post-partum* and lower concentration about 250 days *post-partum*, when compared with cows that calved in autumn. There was an interaction ( $p < 0.05$ ) between SOC and time of year, where herds that calved in spring produced milk with higher concentrations of K in October and January and lower concentrations in April and May–June (Table 4).

The concentrations of Na and K in milk did not vary with the estimated ME content of pasture consumed, the feeding of macro-mineral supplements, or with the amounts of concentrates or conserved forages fed. While the concentration of K in milk increased ( $p < 0.05$ ) by  $2 \pm 0.8$   $\text{mg}/\text{kg}$  for every extra kg DM pasture offered, that of Na was not affected.

The ratio of Na to K varied with time of year declining from 25 to 50 days *post-partum* and increasing from around 150 to 300 days *post-partum* for both calving groups (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and SOL where the ratio of Na to K was higher around 225 and 300 days *post-partum* for herds that calved in spring (Table 4). There was an interaction ( $p < 0.05$ ) between SOC group and time of year, where herds that calved in spring produced milk with a higher ratio of Na to K in April and May–June and lower ratios in October, November–December and January when compared to herds that calved in autumn (Table 4). The ratio of Na to K declined by  $-0.0004 \pm 0.00019$   $\text{g}/\text{g}$  for every extra kg DM pasture offered ( $p < 0.05$ ) and did not vary significantly with the estimated ME content of pasture, the feeding of macro-mineral supplements, or with the amounts of concentrates or conserved forages fed.

### 3.5. Associations between Milk Components

Every extra g/kg of crude protein in milk was associated with increases ( $p < 0.001$ ) in the concentrations of Ca ( $20 \pm 3.0$  mg/kg), Mg ( $2 \pm 0.3$  mg/kg), P ( $17 \pm 2.3$  mg/kg), S ( $8 \pm 1.0$  mg/kg), Se ( $0.7 \pm 0.16$  µg/kg), and Zn ( $0.1 \pm 0.02$  mg/kg), while the concentration of K decreased ( $p < 0.001$ ) by  $22 \pm 5.9$  mg/kg.

The significance of correlations between milk concentrations of various minerals is summarized in Table 7. On average, the ratio of Ca relative to P varied by time of year being higher ( $p < 0.05$ ) in April, May–June, January and February–March when compared with July and August–September. There was no effect of feeding macro-mineral supplements (data not shown) on this ratio. The concentration of citrate in milk was positively correlated ( $p < 0.05$ ) with that of Ca, while there were no significant correlations with the concentrations of other minerals. The concentration of Na in milk was negatively correlated ( $p < 0.05$ ) with that of K.

**Table 7.** Correlations between milk mineral concentrations. Correlations  $\leq -0.40$  and  $\geq 0.40$  are significant ( $p < 0.05$ ) and are in bold.

	Ca	Citrate	K	Mg	Na	P	S	Se
Ca								
Citrate	<b>0.48</b>							
K	NS	NS						
Mg	<b>0.62</b>	NS	NS					
Na	<b>0.51</b>	NS	<b>−0.53</b>	<b>0.52</b>				
P	<b>0.48</b>	NS	NS	NS	NS			
S	<b>0.61</b>	NS	NS	<b>0.58</b>	<b>0.40</b>	<b>0.62</b>		
Se	NS	NS	NS	NS	NS	NS	NS	
Zn	NS	NS	<b>−0.41</b>	NS	NS	NS	NS	NS

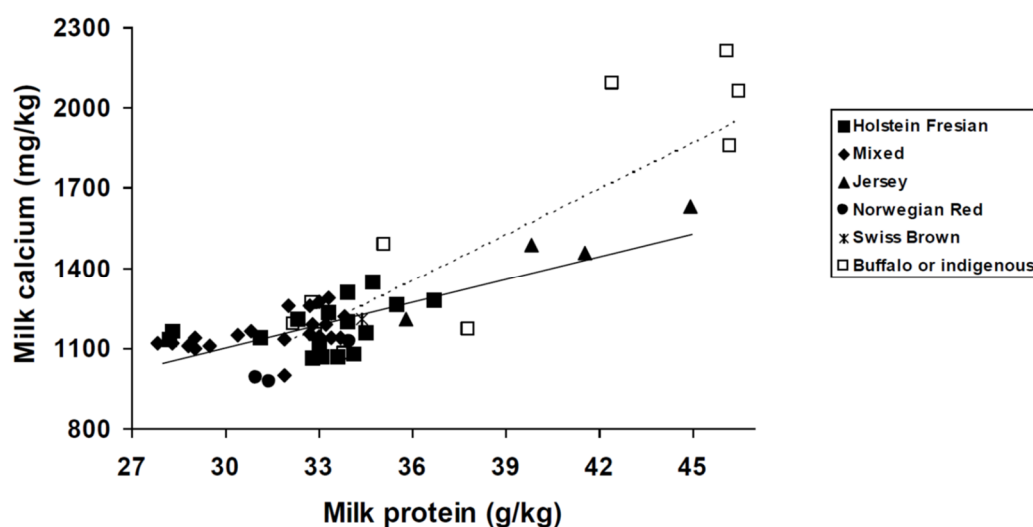
An analysis of the relationship between milk Ca and protein from 22 studies conducted around the world and published since 1978 in dairy cattle, indigenous cattle, and buffalo reveals a strong relationship, given the number of possible confounding factors (Figure 2). Milk Ca increased with increasing milk protein content with the response being similar for all dairy breeds, but with a higher slope for the buffalo and indigenous cattle breeds. Within the dairy breeds, both milk Ca and milk protein were higher in Jerseys than in the other breeds, while buffalo had similar milk protein but higher milk Ca than Jerseys.

## 4. Discussion

Milk and dairy products are an important source of Ca and P and a significant source of Mg in the Australian diet [26,27], and the concentrations of these elements has implications in processing [28,29]. The average concentrations of Ca, P, and Mg from these northern Victorian farms was similar to values reported in Reference [30], who reported mean values for Ca 1% higher, P 3% lower, and Mg 9% higher in milk from herds in New York. In milks sourced from 8 milk outlets, [31] reported Ca concentrations between 1073 and 1372 mg/L and Mg concentrations between 95 and 120 mg/L, the range in Ca concentration being less than in this study. Importantly, all cows sampled in the current study were Holstein Friesian, which is now the predominant breed in Australia, having increased from 38% of the national herd in 1978 to 83% in 2000 [32] and 78% in 2010 [33]. As ~70% of the Ca in milk is bound to protein [34], the low proportion of Jersey, red breeds, and crossbred cows in the national herd likely has an impact on the protein concentrations in milk, as well as the concentrations of minerals, such as Ca, associated with protein. In this context, Reference [35] found that Ca, Mg, and protein concentrations were lower in milk from Holstein Friesians than from Jerseys that were grazed in a single herd.

In the present study, there was an increase in milk Ca with increasing milk protein. Similarly, others have also reported correlations between milk Ca and protein contents [29,36] with similar slopes (~25 mg/kg) to those observed in the present study (20 mg/kg) and generated from the analyses

of published data (28 mg/kg). The mean Ca concentrations in milk from dairy cows reported in a number of studies spanning the period 1978 to 2017 ranged from 975 to 1630 mg/kg (Figure 2), so that the average value observed in this study (1072 mg Ca/kg milk) was at the lower end of this range. Within the dairy breeds, Jersey cows had higher milk Ca concentrations. Excluding these values as being unrepresentative of the predominantly Holstein/Friesian Australian herd, the concentration of Ca in milks from the current study fits approximately midway between values reported from studies largely conducted over the previous 2 decades. The low mean Ca concentrations in the Holstein/Friesian and mixed breed cows (Figure 2) are likely indicative of the emphasis on selection for milk volume in these breeds. However, it is the range in Ca (and other macro element) concentrations in milk, from across these 24 farms in the present study, which were at times quite low, that is of interest to us. Interestingly, very recently, some extremely low levels Ca (ca. 750 mg Ca/kg milk) have been reported in high yielding Polish Holstein-Friesians [37]. Given the increased focus on dairy consumption and effects on Ca status and human health [38,39], it is important that we understand the sources of variation in milk Ca.



**Figure 2.** Relationship between milk calcium and protein contents in cows of various breeds and milking potential. Regression equation is: (Milk calcium) =  $249 \pm 163.0 + 28.4 \pm 4.93 \times$  (Milk protein) for the pooled dairy breed (solid line) –  $1256 \pm 272.03 + 38.0 \pm 7.36 \times$  (Milk protein) for the pooled buffalo and indigenous breeds (dotted line). The  $R^2 = 0.847$  and SE was 108.0 mg/kg for this relationship ( $p < 0.001$ ). There was no effect of breed within the dairy breeds but the intercept was lower ( $p < 0.001$ ) and the slope greater ( $p < 0.001$ ) for the pooled buffalo and indigenous breeds of cattle. References include [31,35,37,40–59] and the present study. Some references may have more than one data point depending upon treatment groups or breeds.

These 24 farms used a split calving system which would reduce seasonal variation in mineral and citrate concentrations of milk supplied to processors, as the bulk milk leaving farms would have concentrations that were described by the weighted average of concentrations in milk from the spring and autumn-calving herds. Some management practices were also found to improve the concentrations of macro elements in milk. For example, the concentrations of Ca, P, and K in milk were higher in cows receiving mineral supplements, with Ca and citrate appearing particularly responsive to the feeding of mineral supplements. As the mineral concentration of grazed herbage and supplements used on dairy farms in northern Victoria vary markedly and are usually unknown [60], the inclusion of a mineral supplement high in Ca and P with cereal grain-based supplements would simply and cheaply correct any issues with low intakes of these elements.

Supplementation with energy rich concentrates is known to increase protein concentration in milk from grazing cows [61]. The concentrations of Ca, P, and Mg in milk increased as the amount of

concentrates fed increased, and there were positive associations between crude protein concentration in milk and the concentrations of Ca, Mg, P, and S. For example, 70% of Ca, 50% of inorganic phosphate, and 30% of Mg are located in the casein micelle [34]. This indicates that nutrition strategies that increase milk casein and protein concentration can also be used to enhance concentrations of key macro-elements in milk. Western diets are often deficient in Mg, and milk and dairy foods offer a means of increasing Mg intake [62], especially given that milk Mg is responsive to supplementation.

The short-term seasonal variations in the relative amounts and forms of Ca, Mg, citrate, and phosphate in milk noted in this study may influence the manufacture of powders and milk concentrates by changing heat stability of milk, cheese manufacture by altering curd firmness, and syneresis capacity of milk (see reviews by [9,11]). In particular, changes in the concentration of citrate in milk affect milk-processing characteristics through its effects on buffering against changes in pH, its role in stabilizing milk against flocculation during heating and freezing by forming complexes with Ca and Mg ions [63,64] and its fermentation products yield distinct aromatic flavor characteristics of fermented milk products [65]. Garnsworthy et al. [66] found that milk citrate was lower mid-lactation than in early-lactation, with late-lactation being intermediate. These authors argued that variation in milk citrate with stage of lactation was related to de novo synthesis of fatty acids (as indicated by a negative correlation between citrate and milk short and medium chain fatty acids (SMCFA)) and that the relationship is independent of diet and milk yield. In the present study there was a weak ( $p < 0.10$ ) negative correlation between average milk citrate and SMCFA concentrations reported in [2].

Milk and dairy products can be a significant source of Se and Zn in the Australian diet. For example, based on this study, milk from northern Victoria would provide women between 19 and 50 years of age consuming about 930 g/day or about 900 mL/day about 19% of the recommended dietary intake for Se and 40% of the recommended dietary intake for Zn. Importantly, the variation in the concentration of Se in milk was much greater than that for Zn.

With Se, it was apparent that management strategies on farms, such as time of calving and feeding concentrate and mineral supplements, can be used to manipulate concentrations in milk. There were large variations in the concentrations of Se in milk between collection periods, that were not fully explained by the effects of SOL. It is likely that the trace elements in mineral supplements included inorganic and/or organic forms of Se and that the concentrates fed varied in Se concentrations. Milk concentrations of this element are increased more by organic than inorganic Se supplements [67] due to greater bioavailability of the former. Concentrations in milk peak quite quickly, within 1 week, of introducing organic Se supplements into the diet [68–70] and increase in a dose-dependent manner [69,71,72]. In addition to responses in milk concentrations from supplementation with selenized yeast, [69] have shown Se concentration in milk of grazing cows is affected by its concentrations in grain supplements. Understanding the causes of variation in milk Se concentration is particularly important to companies producing infant formulae, where the specifications in relation to trace element concentrations are stringent. It is also important to understand the manner in which milk Se responds to dietary Se on farm for the production of dairy products that exhibit physiological functional properties for human health [70]. In this context, dairy protein bound Se is more bioavailable and efficacious than inorganic or yeast protein Se [73].

The management practices across these farms had no significant effect on the concentration of Zn in milk, despite the near 2-fold range between minimum and maximum values observed. In milk collected from 3 manufacturing and 5 retail outlets at 3-week intervals in Ireland, there was little variation in Zn concentration with minimum concentrations of 3.52 mg/L and maximum concentrations of 4.54 mg/L [31]. The average concentrations across all samples were about 10% lower than those reported by Reference [31]. Other work also suggests that the concentration of Zn in milk does not vary significantly with season and is only marginally affected by the concentration of Zn in diet [74]. However, others have found that milk Zn is responsive to dietary Zn sulphate or methionine [75]. Importantly, we found a positive association between milk protein and Zn concentrations, indicating that strategies that increase protein in milk may also increase Zn



concentration. It is perhaps not surprising, then, that dairy supplementation can increase Zn status and protein intake in elderly patients [76] and dietary strategies to increase milk Zn may benefit community health status and immune function.

## 5. Conclusions

Our study indicated that dairy farmers can influence the concentrations of the macro-minerals and citrate as well as Se and Zn concentrations in milk through the calving system they employ. For example, milk produced by herds that calved in autumn had different concentrations of minerals to that from herds that calved in spring, with these differences most apparent in early and late lactation. The concentrations of minerals and citrate in bulk milk leaving these 24 farms were described by the weighted average of milk from the spring- and autumn-calving herds and indicate that split calving reduces seasonal variation in the concentrations of minerals and citrate in milk. Milk Ca concentration was low possibly because of its strong association with milk protein and the low protein content of milk from predominantly Holstein cattle. There is also potential to reduce short term seasonal variation in the concentrations of milk minerals by changing calving patterns and nutritional management on farms. Since the study, there have been large changes to the milk production systems on farms in the region, and it is likely that calving and/or feeding practices have changed. This illustrates the importance of milk processors to understand the production systems (including changes) of their suppliers.

**Author Contributions:** F.R.D., G.P.W. and P.T.D. conceived and designed the study; G.P.W. performed the experiments; F.R.D. and G.P.W. analyzed the data; R.W. conducted protein and mineral analysis; F.R.D., G.P.W. and P.T.D. wrote the paper.

**Funding:** The Department of Economic Development, Jobs, Transport and Resources and Dairy Australia funded this work.

**Acknowledgments:** We acknowledge the capable technical support provided by Daryl Wilson, Pam Guerra (Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources), Maike van der Maat and Irene Van Dorp (Wageningen University, The Netherlands). We are also grateful to Robin Green for statistical advice.

**Conflicts of Interest:** The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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